22 Sirtuin and Resveratrol

Antoni Camins[1,2], *Carme Pelegri*[2,3], *Jordi Vilaplana*[2,3], *Rosa Cristofol*[4], *Coral Sanfeliu*[4], *and Mercè Pallàs*[1,2]

[1]Unitat de Farmacologia i Farmacognòsia i Institut de Biomedicina (IBUB), Facultat de Farmàcia, Universitat de Barcelona, Nucli Universitari de Pedralbes, Barcelona, Spain

[2]Centro de Investigación de Biomedicina en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain

[3]Departament de Fisiologia, Facultat de Farmàcia, Universitat de Barcelona, Nucli Universitari de Pedralbes, Barcelona, Spain

[4]Department d'Isquèmia Cerebral I Neurodegenració, Institut d'Investigacions Biomèdiques de Barcelona, CSIC-IDIBAPS, Barcelona, Spain

CONTENTS

22.1 Introduction 329
22.2 SIRT1 and Neuroprotection 331
22.3 Resveratrol 331
22.4 Resveratrol and Huntington's Disease 333
22.5 Resveratrol and Alzheimer's Disease 334
22.6 Resveratrol and Parkinson's Disease 334
22.7 Resveratrol in Stroke and Brain Damage 335
22.8 Resveratrol and Aging 335
22.9 Hormesis: Axis between SIRT1 and Resveratrol? 336
22.10 Summary 336
Acknowledgments 337
References 337

22.1 INTRODUCTION

Protein acetylation is involved in the regulation of several cellular functions such as protein-protein interactions, protein stability, and DNA recognition by proteins. For instance, the acetylation of histone proteins alters gene transcription [1]. Thus, removal of acetyl groups from lysine residues results in compaction of chromatin and, hence, repression of gene transcription. The process of acetylation and deacetylation regulated by proteins with acetyltransferase activity is important for cellular processes. These proteins are usually known as histone acetyltransferases [2]. Nowadays, there is a growing interest for histone deacetylases (HDACs) because of their potential clinical applications [3]. HDACs have been divided into four groups. Class I and class II HDACs are similar to the yeast Rpd3p and Hda1p proteins. Class III HDACs are similar to the yeast transcriptional repressor Sir2p and are referred to as sirtuins. Class I and class II HDACs are characterized by

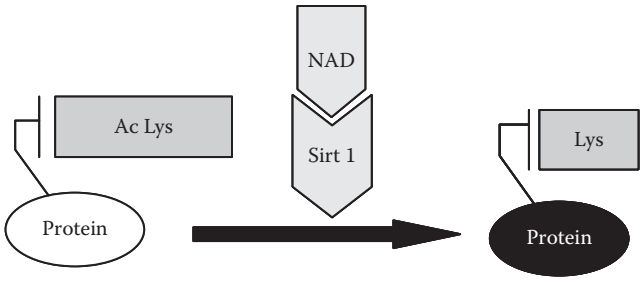

FIGURE 22.1 Enzymatic activity of SIRT1, using NAD^+ as a cofactor, producing a deacetylated substrate and nicotinamide.

their sensitivity to inhibition by trichostatin A, while class III HDACs are dependent on nicotinamide adenine dinucleotide (NAD). Class IV HDACs include the deacetylase HDAC11. The focus of this chapter is on sirtuins (class III HDAC), which are a conserved family of NAD^+-dependent deacetylases and named after the founding member, *Saccharomyces cerevisiae* silent information regulator 2 (Sir2) protein [4]. Its products function in a complex as transcriptional repressors or silencers, acting largely through histone deacetylation, at the telomeres, mating-type loci, and the rDNA gene loci [5] . The *SIR2* gene, required for silencing of rDNA loci, is evolutionarily conserved from prokaryotes to humans. Sir2 is an NAD-dependent class III protein deacetylase [6,7], with ADP-ribosyltransferase activity in vitro. Analysis of SIRT1 enzymatic activity has revealed that it functions differently from previously described HDACs. Studies using purified SIRT1 revealed that for every acetyl lysine group removed, one molecule of NAD is cleaved, and nicotinamide and *O*-acetyl-ADP-ribose are produced (Figure 22.1). Therefore, SIRT1 appears to possess two enzymatic activities: the deacetylation of a target protein and the metabolism of NAD [8,9]. These two activities suggest that SIRT1 could act as a metabolic or oxidative sensor, regulating cellular machinery based on such information.

The need for NAD in the deacetylase activity of SIRT1 has led to the suggestion that enzymatic activity could be regulated by the concentration of NAD, the ratio NAD/NADH, or by the intracellular concentration of nicotinamide [10].

Studies performed in yeast showed that Sir2 deletion leads to histone hyperacetylation. Subsequent studies were focused on this enzyme as a key mediator of *S. cerevisiae*'s replicative life span [10–12].

With reference to the mammalian Sir2 gene family (or sirtuins), seven homologues (SIRT1-7) have been characterized until now, among them the nuclear SIRT1, which is the closest homologue to Sir2, based on amino acid identity, and the best understood in terms of cellular activity and functions. Among the nonhistone cellular substrates of SIRT1, tumor suppressor p53, the transcription factor nuclear factor–kappa B (NF-κB), and the FOXO family of transcription factors have been identified. All of them are involved in the transcriptional control of key genes in cell proliferation and cell survival. Moreover, SIRT1 also deacetylates the nuclear receptor peroxisome-proliferator activated receptor-γ (PPARγ) and its transcriptional coactivator, PPARγ coactivator-α (PGC-α), which regulates a wide range of metabolic activities in muscle, adipose tissues, and liver, linked to hepatic nuclear factor1 (HNF-4α). SIRT1 substrates, therefore, have apparent functions that can link nutrient availability and energy metabolism to adaptive changes in transcriptional profiles that affect cell survival in multiple systems.

Sirtuins are currently an object of interest in various fields of aging medicine, ranging from oncology to gerontology, because of their role as a longevity factor in multiple model organisms. The interest in SIRT1 has also intensified over the past 2 years with further discoveries of its role in cancer, metabolic diseases, and neurodegenerative disorders. The main focus of this review shall

be SIRT1's role in neuronal aging and neurodegenerative diseases, and its possible modulation by a natural activator, resveratrol.

22.2 SIRT1 AND NEUROPROTECTION

Several important roles of SIRT1 have been described in the central nervous system (CNS), mainly in neuronal development and neuroprotection. It is known that there are high levels of SIRT1 expression in the heart, brain, spinal cord, and dorsal root ganglia [13]. Previous studies demonstrated that high SIRT1 levels in the embryonic brain suggest that it might have a role in neuronal and/or brain development. This notion is in agreement with some of the phenotypes associated with SIRT1 knockout mice, in which postnatal survival is infrequent and which have developmental defects [14,15].

As in other mammalian cells, SIRT1 promotes survival and stress tolerance in CNS neurons. However, data in this regard are scarce, because earlier studies have not used neuronal cells. In the adult rat brain, SIRT1 can be found in the hippocampus, cerebellum, and cerebral cortex.

Interestingly, SIRT1 expression is regulated by oxidative stress because the antioxidant vitamin E has been shown to reduce both the oxidative damage and the reduction of SIRT1 caused by a high fat and sugar diet, with the restoration of SIRT1 levels [16]. This study suggests that SIRT1 levels in the brain are affected by oxidative stress and energy homeostasis. A role for SIRT1 in the protection of cardiac myocytes against ischemia-induced apoptosis has been well documented [17,18]. A recent interesting study using organotypic hippocampal slice culture as an in vitro model of cerebral ischemia showed that pretreatment using resveratrol, an activator of SIRT1, mimics ischemic preconditioning via SIRT1 [19]. When SIRT1 is inactivated by sirtinol after ischemic preconditioning or resveratrol pretreatment, neuroprotection is abolished. This study demonstrated a neuroprotective role of SIRT1 in ischemic injury, which could be elicited by a small molecule such as resveratrol, and it is therefore of substantial clinical interest. In contrast, another earlier report had shown that the sirtuin inhibitor nicotinamide enhances neuronal cell survival in acute anoxic injury [20], although a clear involvement of SIRT1 in this case was not clearly demonstrated.

22.3 RESVERATROL

Resveratrol (3,5,4′-trihydroxystilbene) was first isolated from the roots of white hellebore (*Veratrum grandiflorum* O. Loes) in 1940 and later, in 1963, from the roots of *Polygonum cuspidatum*, a plant used in traditional Chinese and Japanese medicine. Initially characterized as a phytoalexin, resveratrol, a polyphenol present in black grapes and its derivatives, attracted little interest until 1992, when it was postulated to account for some of the cardioprotective effects of red wine. Since then, dozens of reports have shown that resveratrol can prevent or slow down the progression of a wide variety of illnesses, including cancer, cardiovascular disease, and ischemic injuries, as well as enhance stress resistance and extend the life span of various organisms from yeast to vertebrates [21,22]. Recent reports indicate that resveratrol treatment alone has a range of beneficial effects in mice, but does not increase the longevity of ad libitum-fed animals when started midlife in contrast to high-fat diet–fed mice [22–24]. Chapter 24 discusses resveratrol properties in greater detail.

The mechanism by which resveratrol exerts such a range of beneficial effects across species and disease models is not yet clear [25], although at the beginning it was proposed that the antioxidant properties of this drug may explain the majority of its beneficial effects. Attempts to show its favorable effects *in vitro* have met with almost universal success, and have led to the identification of multiple direct targets for this compound. However, results from pharmacokinetic studies indicate that circulating resveratrol is rapidly metabolized, and cast doubt on the physiological relevance of the high concentrations typically used for in vitro experiments [26,27]. Further experiments are needed to show whether resveratrol or its metabolites accumulate sufficiently in tissues to recapitulate in vitro observations, or whether alternative higher-affinity targets, such as quinone reductase 2, have the key roles in its protective effects [28,29]. In vivo results have, therefore, become increasingly

important in the attempts to understand how effective resveratrol is in the treatment of different diseases. It is also unclear what conclusion should be drawn from the studies described so far. The benefits of resveratrol, as we noted above, can be explained by its antioxidant properties or better if this substance acts through a specific genetic pathway that has evolved to increase disease and stress resistance. With regard to the latter proposal, there is already an ample evidence for the existence of health-promoting pathways that are activated by caloric restriction. It has been known since the 1930s that a severe lowering of caloric intake dramatically slows down the rate of aging in mammals and delays the onset of numerous diseases of aging, including cancer, cardiovascular disease, diabetes, and neurodegeneration. The hypothesis that resveratrol might use the same pathways activated by caloric restriction in mammals is attractive because it appears to do so in lower organisms; however, proving this hypothesis will require a better understanding of these processes.

In reference to antioxidant action of resveratrol, it is widely accepted that resveratrol exerts antioxidant effects, but it is not yet clear if this is primarily a direct scavenging effect or the result of the activation of pathways that up-regulate cells' natural antioxidant defenses. Reactive oxygen species (ROS) have been shown to have a role in the initiation and progression of cancer by directly damaging DNA and other macromolecules. In addition to its possible modulation of antioxidant enzymes involved in the phase II response, resveratrol has an intrinsic antioxidant capacity that could be related to its chemopreventive effects. In vivo, resveratrol has been shown to increase plasma antioxidant capacity and decrease lipid peroxidation; however, it is difficult to assess whether these effects are direct or the result of up-regulation of endogenous antioxidant enzymes. In addition, clinical trials of antioxidant molecules have yielded disappointing results, suggesting that phytochemicals could possess other properties that are more relevant to cancer prevention. Oxidation of low-density lipoprotein (LDL) particles is strongly associated with the risk of coronary heart disease and myocardial infarction. Resveratrol prevents LDL oxidation in vitro by chelating copper, as well as by directly scavenging free radicals (although other components of red wine are superior free radical scavengers) [30]. Treatment of normal rats with resveratrol does not affect lipid peroxidation, as reflected by the presence of thiobarbituric acid-reactive substances [31]. However, resveratrol can be detected in LDL particles from humans after consumption of red wine, which is rich in this compound, and the pure compound prevents increases in lipid peroxidation induced by tumors or ultraviolet irradiation [32,33], in addition to blocking gentamicin-induced nephrotoxicity [34]. In stroke-prone, spontaneously hypertensive rats, resveratrol significantly reduces markers of oxidative stress such as glycated albumin in serum, and 8-hydroxyguanosine in urine [35]. Furthermore, in guinea pigs, resveratrol induces the activities of QR1 and catalase in cardiac tissue, and decreases the concentration of ROS generated by menadione [36]. These results indicate that resveratrol can suppress pathological increases in the peroxidation of lipids and other macromolecules in vivo, but whether the mechanism is direct, indirect, or both is not yet clear.

Another mechanism by which resveratrol could combat tumor formation is induction of cell cycle arrest and apoptosis. The antiproliferative and proapoptotic effects of resveratrol in tumor cell lines have been extensively documented in vitro and are supported by down-regulation of cell cycle proteins and increases in apoptosis in tumor models in vivo. Although resveratrol has been found to target leukemic cells preferentially in vitro in some studies, the specificity of these effects remains unclear because other researchers have found that resveratrol inhibits growth and induces apoptosis in normal hematopoietic cells at similar doses. Some level of specificity could arise from the apparent increased susceptibility of cycling cells to the effects of resveratrol [37]. A more precise mechanism by which resveratrol could act is sensitization of tumor cells to other inducers of apoptosis. Resveratrol has been shown to sensitize several tumor lines, but not normal human fibroblasts, to TRAIL (tumor necrosis factor–related apoptosis-inducing ligand)-induced apoptosis. It remains to be seen whether the proapoptotic effects of resveratrol in vivo are related to these in vitro observations, or secondary to other effects, such as inhibition of angiogenesis.

The last protective mechanism related with resveratrol is its role as activator of SIRT1. Resveratrol increases the affinity of SIRT1 for its acetylated substrates, possibly inducing a conformational

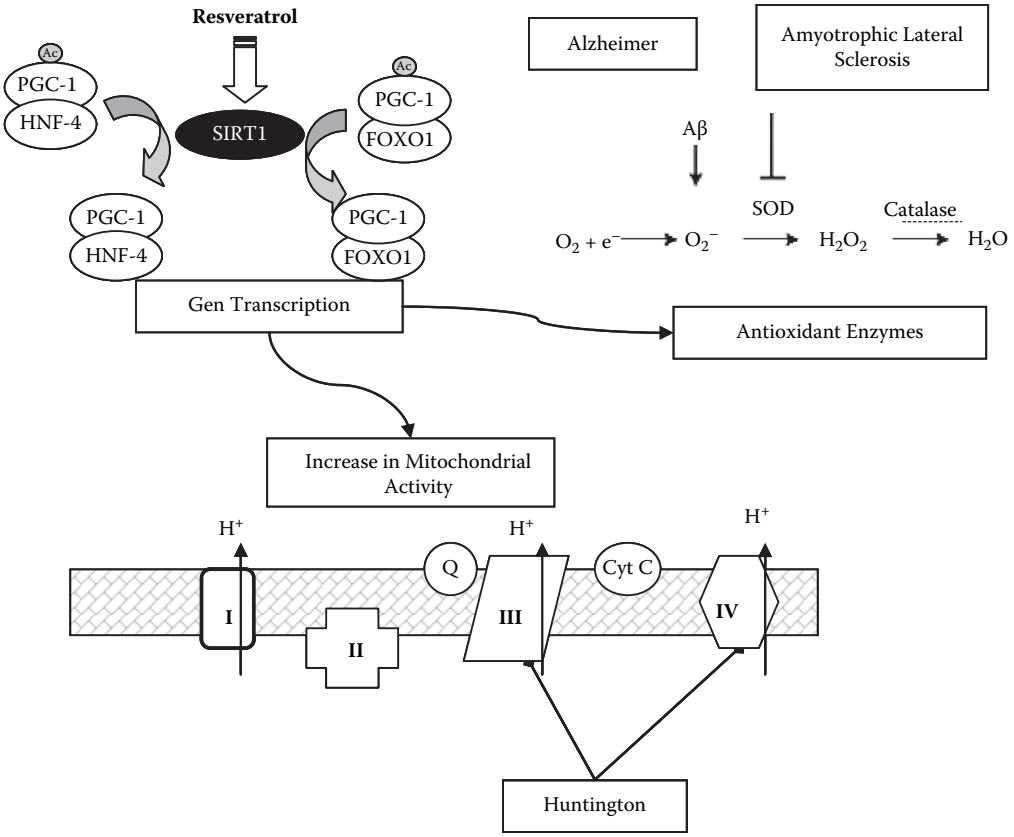

FIGURE 22.2 Scheme illustrating the effect of resveratrol on mitochondrial biogenesis and its influence in the antioxidant enzymes gene transcription through the SIRT1/PGC-1α/FOXO pathway. Main points of dysfunction for some neurodegenerative diseases are also indicated.

change of SIRT1 [38]. The rat brain has receptors for polyphenols such as resveratrol. This indicates that this substance and its derivatives can pass the blood-brain barrier, and several studies suggest that it may have a protective effect in some neurodegenerative processes, as we will describe below in greater extent. Hence, the axis SIRT1/PGC-1 activated by resveratrol (Figure 22.2) is a signaling pathway involved in several cellular contexts and each of the actors involved may promote a separate slowdown in the neurodegenerative process [39]. This neuroprotective action is very likely because the central factor of this signal, PGC-1, promotes mitochondrial activity, while neurodegenerative diseases are linked to mitochondrial failures. It is strongly suggested that the activation of the axis SIRT1/PGC-1 by resveratrol could be a key feature of the mechanisms of neuroprotection by this polyphenol and could lead to new therapeutic prospects (Figure 22.2).

22.4 RESVERATROL AND HUNTINGTON'S DISEASE

Using the neurotoxin 3-nitropropionic acid, a mitochondrial complex II inhibitor, and a well-established experimental model of Huntington's disease, it has been reported that the beneficial effects of resveratrol against this neurotoxin might be attributed to its antioxidant activity [40]. However, several findings have converged on the notion that SIRT1's neuroprotective effect could be extended to degenerating neurons. Parker and coworkers [41] showed that resveratrol, acting through Sir2 and SIRT1 activation, respectively, protected *Caenorhabditis elegans* and mouse neurons against the cytotoxicity of the mutant polyglutamine protein huntingtin. Huntingtin is the

product of the gene mutated in the hereditary neurodegenerative disorder Huntington's disease, whose expansion of a polyglutamine stretch resulted in a mutant polypeptide that could form cytotoxic aggregates in neurons [42]. Although *C. elegans* has no huntingtin orthologue, overexpression of a huntingtin fragment in touch receptor neurons resulted in a gain-of-function mechanosensory defect that could model the disease. Both resveratrol and an increased sir-2.1 gene dosage alleviated the worm neuronal dysfunction in a DAF16-dependent manner. Furthermore, resveratrol decreased cell death associated with neurons cultured from a mutant huntingtin (109Q) knocking mice, in a manner that is reversible by two SIRT1 inhibitors, sirtinol and nicotinamide [43].

22.5 RESVERATROL AND ALZHEIMER'S DISEASE

A link between SIRT1 and Alzheimer's disease (AD) is also becoming increasingly evident [44,45]. The amyloid hypothesis [46] depicts that extracellular plaques consist of aggregated beta-amyloid (Aβ) peptide generated from proteolytic cleavages of the amyloid precursor protein (APP) as the etiological agent of AD pathology [47]. Both intracellular and extracellular soluble oligomeric forms of Aβ could, in fact, initiate synaptic malfunctions and the onset of AD symptoms [48,49]. NF-κB signaling in microglia is known to be critically involved in neuronal death induced by Aβ peptides [50]. Chen and collaborators [51] showed that stimulation of microglia with Aβ increased acetylation of RelA/p65 subunit of NF-κB at lysine 310. Overexpression of SIRT1 and resveratrol treatment markedly reduced NF-κB signaling stimulated by Aβ and had strong neuroprotective effects. This result connects the known role of SIRT1 in modulating NF-κB activity [52] with AD. It should be kept in mind that, for AD, as with other neurodegenerative diseases, the beneficial effect of resveratrol is multifaceted. Its immediate effect is more likely associated with its activity as an antioxidant [53,54], but at a more extended time frame, its activation of SIRT1 and modulation of NF-κB signaling may result in other beneficial effects, such as anti-inflammation.

Another possible link between SIRT1 and AD came from the potential benefits of calorie restriction (CR) on AD symptoms and progression. It is well known in the epidemiology of neurodegenerative diseases that the incidence of sporadic Parkinson's disease (PD) and AD are both correlated with multiple genetic factors, diet, and social behavior [55]. High calorie diets are associated with the risk of AD, and CR has been proposed to protect against both PD and AD[56]. Firmer evidence for this idea was obtained when Patel [57] showed that short-term CR substantially decreased the accumulation of Aβ plaques in two AD-prone APP/presenilin transgenic mice lines, and also decreased gliosis marked by astrocytic activation. In another study, Wang and colleagues [58] also showed that a CR dietary regimen prevents Aβ peptide generation and neuritic plaque deposition in the brain of another mouse model of AD (Tg2576 mice). In this latter study, the authors suggested that CR resulted in the promotion of APP processing via the nonamyloidogenic α-secretase–mediated pathway. They observed a larger-than-twofold increase in the concentration of brain sAPPα (a product of α-secretase cleavage) and a statistically significant 30% increase in ADAM10 (a putative α-secretase) levels in CR animals compared to controls. There also appeared to be a moderate increase in the levels of the insulin degrading enzyme, which has been associated to brain amyloid clearance [59]. In another recent report, the same group showed that CR resulted in reduced contents of Aβ in the temporal cortex of squirrel monkeys, in a manner that was inversely correlated with SIRT1 protein concentrations in the same brain region [60]. It is not particularly clear in the above reports whether CR's effects in attenuating amyloid production were mediated through SIRT1 activation. Recent evidence suggests that this may indeed be the case, and may actually involve a novel signaling crosstalk [61].

22.6 RESVERATROL AND PARKINSON'S DISEASE

PD is a neurodegenerative disease that is also characterized at the clinical level by bradykinesia, tremor, and rigidity, and at the cellular level by a loss of dopamine neurons of the gray matter and the frequent presence of intraneuronal inclusions named Lewy bodies, mainly composed of fibrillar

α-synuclein [62]. Like AD, the familial form of PD concerns only a small proportion of patients (10%). The majority of them are suffering from a sporadic form and, if the genetic causes are fairly well identified, the reasons for the emergence of the sporadic contrary are still unclear. The involvement of mitochondrial dysfunction in the PD has been established for more than two decades when it was discovered that the administration of 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine causes the emergence of parkinsonism in laboratory animals and also in humans, through its active metabolite ion MPP^+, which inhibits the complex I of the chain of mitochondrial electron transport. It is well known that complex I is the major source of production of free radicals; the assumption is that the alteration of its functions could, beyond the declining production of adenosine triphosphate, give rise to increased oxidative stress, explaining the emergence of the disease. However, different authors working on different PD models conclude that SIRT1 activation does not play a major role in the protective effect of resveratrol against MPP^+ cytotoxicity, because sirtuin inhibitors such as nicotinamide and sirtinol did not counteract neuroprotection by resveratrol [63]. Instead, all works point to propose that antioxidative actions are responsible for neuroprotection by resveratrol against MPP^+ [63,64]. However, it has been recently described that genetic inhibition of SIRT2 via small interfering RNA rescued α-synuclein toxicity [65]. Furthermore, the inhibitors of this enzyme protected against dopaminergic cell death both in vitro and in a *Drosophila* model of PD, and found that inhibition of SIRT2 rescued α-synuclein toxicity and modified inclusion morphology in a cellular model of PD [65]. However, increased SIRT1 expression or activity delays the toxic effects induced by α-synuclein, the protein that forms insoluble aggregates in several age-onset pathologies including PD. Resveratrol could be an interesting candidate for potential application in the treatment of PD only by its antioxidant properties [63].

22.7 RESVERATROL IN STROKE AND BRAIN DAMAGE

Numerous studies have raised the possibility that resveratrol might be useful in protecting against brain damage following cerebral ischemia. Laboratory animals given intraperitoneal injections of resveratrol showed less motor impairment and significantly smaller infarct volume jointly with decreased delayed neuronal cell death and glial cell activation after ischemia. Similar effects were observed in wild-type, but not peroxisome proliferators–activated receptor-$\alpha^{-/-}$, mice [66]. Resveratrol administered intraperitoneally also prevented seizures induced by $FeCl_3$, kainic acid [67] or pentylenetetrazole [68], and partially restored cognition in rats receiving streptozotocin intracerebroventricularly [69]. These results indicate that resveratrol is capable of penetrating the blood-brain barrier and exerts strong neuroprotective effects, even at low doses, after stroke or on neurotoxin-injured brain. Moreover, more studies are necessary to determine if these neuroprotective effects are mediated trough the stimulation of SIRT1 or by its antioxidant properties [21].

22.8 RESVERATROL AND AGING

As mentioned, in yeast, worms, and flies, extra copies of genes encoding sirtuins are associated with extended life span [11,70,71]. Inbred knockout mice that lack SIRT1 show developmental defects, have a low survival rate, and have a significantly shorter life span compared to wild-type mice, although out breeding seems to improve the phenotype significantly [15]. It has been postulated that the main function of sirtuin proteins might be to promote survival and stress resistance in times of adversity [72]. An evolutionary advantage arising from the ability to modify life span in response to environmental conditions could have allowed these enzymes to be conserved as species evolved, and to take on new functions in response to new stresses and demands on the organism. This could explain why the same family of enzymes has dramatic effects on life span in different organisms with seemingly dissimilar causes of aging [73]. Caloric restriction and intermittent fasting are implicated in most of the theories for successful brain aging [74].The data from lower organisms have

provoked intense research into the function of sirtuin proteins in mammalian systems. An in vitro screen for activators of SIRT1 identified resveratrol as the most potent of 18 inducers of deacetylase activity [12]. Subsequent work has shown that resveratrol extends the life span of *S. cerevisiae*, *C. elegans*, and *Drosophila melanogaster*, but only if the gene that encodes Sir2 is present in these organisms. More recently, resveratrol was shown to extend the maximum life span of a species of short-lived fish by up to 59%, concomitant with the maintenance of learning and motor function with age and a dramatic decrease in aggregated proteins in elderly fish brains [75]; however, the extent to which this effect is Sir2-dependent, if at all, is not known. Moreover, resveratrol consistently recapitulates the protective effects of SIRT1 overexpression in cell culture, and Sir2/SIRT1 have been shown as essential mediators of effects on adipogenesis, NF-κB acetylation, protection from mutant huntingtin protein, and life span extension in lower organisms [11,41,70,76].The question of whether enhanced SIRT1 activity and/or resveratrol treatment will increase mammalian life span looms large in the aging-research community.

22.9 HORMESIS: AXIS BETWEEN SIRT1 AND RESVERATROL?

"Hormesis" describes the phenomenon in which a mild stress (e.g., irradiation, heat, or toxins) can induce a protective response against subsequent stresses [77]. This hormetic response is credited for the paradoxical result that mildly stressed animals outlive their unstressed counterparts, which also possibly applies to humans. It has been suggested that caloric restriction then activation of SIRT1 might act as a mild stress to induce a hormetic response [78], which could account for enhanced stress tolerance and longevity in calorie-restricted mice, as well as the otherwise counterintuitive finding that such animals are better able to resist starvation [79]. In yeast, at least, this contention is strongly supported by the observation that both caloric restriction and mild stresses induce expression of Pnc1, an upstream activator of sirtuin proteins that is necessary and sufficient for life span extension [80]. The "xenohormesis hypothesis" postulates that sensing stress responses, such as resveratrol accumulation, in a food source might be sufficient to induce a hormetic response in animals eating that food. It can be imagined that throughout evolution, such stress markers in the surrounding vegetation would have served as strong predictors of a coming famine or direct stress to the animal. Reacting to these molecules would allow the hormetic response to begin ahead of any direct damage or energy deficit, and, more importantly, would not stake the life of the animal on the hope that the initial stress would be mild and/or protective. If the xenohormesis hypothesis is correct, then stressed plants might form an abundant reservoir for medicinal compounds that trigger conserved protective responses in humans [81]. The relatively low amounts of resveratrol in foods belie the possibility that there are numerous potential xenohormetic compounds in a stressed plant that could act additively or even synergistically. Indeed, another potentially xenohormetic compound, quercetin, behaves similarly to resveratrol in many assays and also inhibits sulfation of resveratrol, which predicts a greater-than-additive effect.

22.10 SUMMARY

In the past decade, sirtuin biology has traveled a long way from their original description as yeast NAD^+-dependent class III HDACs that control yeast life span. In mammals, seven orthologues of Sir2 have been identified, SIRT1 to SIRT7, and the exact biological function of most of these sirtuins still remains only partially characterized. Of particular interest is the fact that SIRT1 not only deacetylates histones to mediate gene silencing, but is also able to interact with and deacetylate some well-known transcriptional regulators, thereby modulating specifically various biological processes. Hence, modulating the expression of SIRT1 or its activity, by using sirtuin activating compounds such as resveratrol, will have pleiotropic effects. SIRT1 activation reduces fat accumulation and adipocyte differentiation through repression of the activity of the adipogenic nuclear receptor PPARγ. SIRT1 also promotes mitochondrial function and energy expenditure and consequently protects

mice from diet-induced obesity, through deacetylation and subsequent activation of PGC-1a in the skeletal muscle and in the brown adipose tissue. The SIRT1/PGC-1a interaction is also important in the liver, where SIRT1 activation upon fasting induces gluconeogenesis and prevents against hepatosteatosis. In addition, SIRT1 significantly enhances insulin secretion in the pancreatic β cells. In combination, these studies illustrate that SIRT1 is a major modulator of metabolism. SIRT1 activation also seems to be endowed with neuroprotective activities, as suggested from the study of models of Huntington disease or AD (Figure 22.2). Furthermore, other sirtuins might play important roles in some diseases, as illustrated by SIRT2, which is down-regulated in human gliomas and could be involved in cancer treatment. Obviously, more studies, in animal models and humans, are still needed to define the exact role of sirtuins in the pathophysiology of human diseases. It can, however, be predicted that therapeutic interventions aiming at activating or blocking sirtuins, depending on the context, will one day become helpful in the treatment of human diseases.

This chapter discusses the effects of SIRT1 modulation by resveratrol that have been observed in vivo and possible evolutionary explanations, as they relate to the development of human therapeutics, based on either resveratrol itself or new, more potent compounds that mimic its effects.

ACKNOWLEDGMENTS

We thank the Language Assessment Service of the University of Barcelona for revising the manuscript. This study was supported by several grants: SAF-2006-13092, BFU-2006-13092 from Ministerio de Educación y Ciencia, PI 041300 from Instituto de Salud Carlos III, 2005/SGR00893from Generalitat de Catalunya, and from Fundació La Marató de TV3 (0310).

REFERENCES

1. Lee, K.K., and Workman, J.L. Histone acetyltransferase complexes: One size doesn't fit all. *Nat. Rev. Mol. Cell Biol.* 8, 284, 2007.
2. An, W. Histone acetylation and methylation: Combinatorial players for transcriptional regulation. *Subcell. Biochem.* 41, 351, 2007.
3. Yang, X.J., and Seto, E. HATs and HDACs: From structure, function and regulation to novel strategies for therapy and prevention. *Oncogene* 26, 5310, 2007.
4. Brachmann, C.B., Sherman, J.M., Devine, S.E. et al. The *SIR2* gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. *Genes Dev.* 9, 2888, 1995.
5. Blander, G., and Guarente, L. The Sir2 family of protein deacetylases. *Annu. Rev. Biochem.* 73, 417, 2004.
6. Denu, J.M. Linking chromatin function with metabolic networks: Sir2 family of NAD(+)-dependent deacetylases. *Trends Biochem. Sci.* 28, 41, 2003.
7. Imai, S., Armstrong, C.M., Kaeberlein, M. et al. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403, 795, 2000.
8. Frye, R.A. Characterization of five human cDNAs with homology to the yeast *SIR2* gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. *Biochem. Biophys. Res. Commun.* 260, 273, 1999.
9. Michan, S., and Sinclair, D. Sirtuins in mammals: Insights into their biological function. *Biochem. J.* 404, 1, 2007.
10. Lin, S.J., Defossez, P.A., and Guarente, L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* 289, 2126, 2000.
11. Kaeberlein, M., McVey, M., and Guarente, L. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms 1. *Genes Dev.* 13, 2570, 1999.
12. Howitz, K.T., Bitterman, K.J., Cohen, H.Y. et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 425, 191, 2003.
13. Sakamoto, J., Miura, T., Shimamoto, K. et al. Predominant expression of Sir2alpha, an NAD-dependent histone deacetylase, in the embryonic mouse heart and brain. *FEBS Lett.* 556, 281, 2004.

14. Cheng, H.L., Mostoslavsky, R., Saito, S. et al. Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 100, 10794, 2003.
15. McBurney, M.W., Yang, X., Jardine, K. et al. The mammalian SIR2alpha protein has a role in embryogenesis and gametogenesis. *Mol. Cell Biol.* 23, 38, 2003.
16. Wu, A., Ying, Z., and Gomez-Pinilla, F. Oxidative stress modulates Sir2alpha in rat hippocampus and cerebral cortex. *Eur. J. Neurosci.* 23, 2573, 2006.
17. Alcendor, R.R., Kirshenbaum, L.A., Imai, S. et al. Silent information regulator 2alpha, a longevity factor and class III histone deacetylase, is an essential endogenous apoptosis inhibitor in cardiac myocytes. *Circ. Res.* 95, 971, 2004.
18. Pillai, J.B., Isbatan, A., Imai, S. et al. Poly(ADP-ribose) polymerase-1–dependent cardiac myocyte cell death during heart failure is mediated by NAD^+ depletion and reduced Sir2alpha deacetylase activity. *J. Biol. Chem.* 280, 43121, 2005.
19. Raval, A.P., Dave, K.R., and Perez-Pinzon, M.A. Resveratrol mimics ischemic preconditioning in the brain. *J. Cereb. Blood Flow Metab.* 26, 1141, 2006.
20. Chong, Z.Z., Lin, S.H., Li, F. et al. The sirtuin inhibitor nicotinamide enhances neuronal cell survival during acute anoxic injury through AKT, BAD, PARP, and mitochondrial associated "anti-apoptotic" pathways. *Curr. Neurovasc. Res.* 2, 271, 2005.
21. Baur, J.A. and Sinclair, D.A. Therapeutic potential of resveratrol: The in vivo evidence. *Nat. Rev. Drug Discov.* 5, 493, 2006.
22. Baur, J.A., Pearson, K.J., Price, N.L. et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444, 337, 2006.
23. Pearson, K.J., Baur, J.A., Lewis, K.N. et al. Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab.* 8, 157–168, 2008.
24. Barger, J.L., Kayo, T., Vann, J.M. et al. A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. *PLoS. ONE.* 3, e2264, 2008.
25. Fremont, L. Biological effects of resveratrol. *Life Sci.* 66, 663, 2000.
26. Walle, T., Hsieh, F., DeLegge, M.H. et al. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab. Dispos.* 32, 1377, 2004.
27. Asensi, M., Medina, I., Ortega, A. et al. Inhibition of cancer growth by resveratrol is related to its low bioavailability. *Free Radic. Biol. Med.* 33, 387, 2002.
28. Hsieh, T.C., Wang, Z., Hamby, C.V. et al. Inhibition of melanoma cell proliferation by resveratrol is correlated with upregulation of quinone reductase 2 and p53. *Biochem. Biophys. Res. Commun.* 334, 223, 2005.
29. Buryanovskyy, L., Fu, Y., Boyd, M. et al. Crystal structure of quinone reductase 2 in complex with resveratrol. *Biochemistry* 43, 11417, 2004.
30. Holvoet, P. Oxidized LDL and coronary heart disease. *Acta Cardiol.* 59, 479, 2004.
31. Turrens, J.F., Lariccia, J., and Nair, M.G. Resveratrol has no effect on lipoprotein profile and does not prevent peroxidation of serum lipids in normal rats. *Free Radic. Res.* 27, 557, 1997.
32. Renaud, S., and de, L.M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* 339, 1523, 1992.
33. Afaq, F., Adhami, V.M., and Ahmad, N. Prevention of short-term ultraviolet B radiation-mediated damages by resveratrol in SKH-1 hairless mice. *Toxicol. Appl. Pharmacol.* 186, 28, 2003.
34. Morales, A.I., Buitrago, J.M., Santiago, J.M. et al. Protective effect of *trans*-resveratrol on gentamicin-induced nephrotoxicity. *Antioxid. Redox. Signal.* 4, 893, 2002.
35. Mizutani, K., Ikeda, K., Kawai, Y. et al. Protective effect of resveratrol on oxidative damage in male and female stroke-prone spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* 28, 55, 2001.
36. Floreani, M., Napoli, E., Quintieri, L. et al. Oral administration of *trans*-resveratrol to guinea pigs increases cardiac DT-diaphorase and catalase activities, and protects isolated atria from menadione toxicity. *Life Sci.* 72, 2741, 2003.
37. Ferry-Dumazet, H., Garnier, O., Mamani-Matsuda, M. et al. Resveratrol inhibits the growth and induces the apoptosis of both normal and leukemic hematopoietic cells. *Carcinogenesis* 23, 1327, 2002.
38. Kaeberlein, M., McDonagh, T., Heltweg, B. et al. Substrate-specific activation of sirtuins by resveratrol. *J. Biol. Chem.* 280, 17038, 2005.
39. Nemoto, S., Fergusson, M.M., and Finkel, T. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{alpha}. *J. Biol. Chem.* 280, 16456, 2005.
40. Kumar, P., Padi, S.S., Naidu, P.S. et al. Effect of resveratrol on 3-nitropropionic acid-induced biochemical and behavioural changes: Possible neuroprotective mechanisms. *Behav. Pharmacol.* 17, 485, 2006.

41. Parker, J.A., Arango, M., Abderrahmane, S. et al. Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons. *Nat. Genet.* 37, 349, 2005.
42. Borrell-Pages, M., Zala, D., Humbert, S. et al. Huntington's disease: From huntingtin function and dysfunction to therapeutic strategies. *Cell Mol. Life Sci.* 63, 2642, 2006.
43. Tang, B.L., and Chua, C.E.L. SIRT1 and neuronal diseases. *Mol Aspects Med* 29, 187, 2008.
44. Anekonda, T.S. Resveratrol—a boon for treating Alzheimer's disease? *Brain Res. Rev.* 52, 316, 2006.
45. Anekonda, T.S., and Reddy, P.H. Can herbs provide a new generation of drugs for treating Alzheimer's disease? *Brain Res. Brain Res. Rev.* 50, 361, 2005.
46. Hardy, J., and Selkoe, D.J. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* 297, 353, 2002.
47. Pallas, M., and Camins, A. Molecular and biochemical features in Alzheimer's disease. *Curr. Pharm. Des* 12, 4389, 2006.
48. Wirths, O., Multhaup, G., and Bayer, T.A. A modified beta-amyloid hypothesis: Intraneuronal accumulation of the beta-amyloid peptide—the first step of a fatal cascade. *J. Neurochem.* 91, 513, 2004.
49. Cuello, A.C. Intracellular and extracellular Abeta, a tale of two neuropathologies. *Brain Pathol.* 15, 66, 2005.
50. Valerio, A., Boroni, F., Benarese, M. et al. NF-kappaB pathway: A target for preventing beta-amyloid (Abeta)-induced neuronal damage and Abeta42 production. *Eur. J. Neurosci.* 23, 1711, 2006.
51. Chen, J., Zhou, Y., Mueller-Steiner, S. et al. SIRT1 protects against microglia-dependent amyloid-beta toxicity through inhibiting NF-kappaB signaling. *J. Biol. Chem.* 280, 40364, 2005.
52. Yeung, F., Hoberg, J.E., Ramsey, C.S. et al. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J.* 23, 2369, 2004.
53. Frank, B., and Gupta, S. A review of antioxidants and Alzheimer's disease. *Ann. Clin. Psychiatry* 17, 269, 2005.
54. Pervaiz, S. Resveratrol: From grapevines to mammalian biology. *FASEB J.* 17, 1975, 2003.
55. Mattson, M.P., Duan, W., Chan, S.L. et al. Neuroprotective and neurorestorative signal transduction mechanisms in brain aging: Modification by genes, diet and behavior. *Neurobiol. Aging* 23, 695, 2002.
56. Mattson, M.P. Will caloric restriction and folate protect against AD and PD? *Neurology* 60, 690, 2003.
57. Patel, N.V., Gordon, M.N., Connor, K.E. et al. Caloric restriction attenuates Abeta-deposition in Alzheimer transgenic models. *Neurobiol. Aging* 26, 995, 2005.
58. Wang, R., Wang, B., He, W. et al. Wild-type presenilin 1 protects against Alzheimer disease mutation-induced amyloid pathology. *J. Biol. Chem.* 281, 15330, 2006.
59. Farris, W., Mansourian, S., Leissring, M.A. et al. Partial loss-of-function mutations in insulin-degrading enzyme that induce diabetes also impair degradation of amyloid beta-protein. *Am. J. Pathol.* 164, 1425, 2004.
60. Qin, W., Chachich, M., Lane, M. et al. Calorie restriction attenuates Alzheimer's disease type brain amyloidosis in squirrel monkeys (*Saimiri sciureus*). *J. Alzheimers. Dis.* 10, 417, 2006.
61. Qin, W., Yang, T., Ho, L. et al. Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer disease amyloid neuropathology by calorie restriction. *J. Biol. Chem.* 281, 21745, 2006.
62. Chen, L., and Feany, M.B. [alpha]-Synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. *Nat. Neurosci.* 8, 657, 2005.
63. Okawara, M., Katsuki, H., Kurimoto, E. et al. Resveratrol protects dopaminergic neurons in midbrain slice culture from multiple insults. *Biochem. Pharmacol.* 73, 550, 2007.
64. Alvira, D., Tajes, M., Verdaguer, E. et al. Inhibition of the cdk5/p25 fragment formation may explain the antiapoptotic effects of melatonin in an experimental model of Parkinson's disease. *J. Pineal Res.* 40, 251, 2006.
65. Outeiro, T.F., Kontopoulos, E., Altmann, S.M. et al. Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. *Science* 317, 516, 2007.
66. Inoue, H., Jiang, X.F., Katayama, T. et al. Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferator-activated receptor [alpha] in mice. *Neuroscience Letters* 352, 203, 2003.
67. Gupta, Y.K., Briyal, S., and Chaudhary, G. Protective effect of *trans*-resveratrol against kainic acid-induced seizures and oxidative stress in rats. *Pharmacol. Biochem. Behav.* 71, 245, 2002.
68. Gupta, Y.K., Chaudhary, G., and Srivastava, A.K. Protective effect of resveratrol against pentylenetetrazole-induced seizures and its modulation by an adenosinergic system. *Pharmacology* 65, 170, 2002.
69. Gupta, Y.K., Chaudhary, G., Sinha, K. et al. Protective effect of resveratrol against intracortical $FeCl_3$-induced model of posttraumatic seizures in rats. *Methods Find. Exp. Clin. Pharmacol.* 23, 241, 2001.

70. Tissenbaum, H.A., and Guarente, L. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* 410, 227, 2001.
71. Rogina, B., and Helfand, S.L. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc. Natl. Acad. Sci. U. S. A.* 101, 15998, 2004.
72. Guarente, L., and Picard, F. Calorie restriction—the SIR2 connection. *Cell* 120, 473, 2005.
73. Koubova, J., and Guarente, L. How does calorie restriction work? *Genes Dev.* 17, 313, 2003.
74. Martin, B., Mattson, M.P., and Maudsley, S. Caloric restriction and intermittent fasting: Two potential diets for successful brain aging. *Ageing Res. Rev.* 5, 332, 2006.
75. Valenzano, D.R., Terzibasi, E., Genade, T. et al. Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. *Curr. Biol.* 16, 296, 2006.
76. Picard, F., and Guarente, L. Molecular links between aging and adipose tissue. *Int. J. Obes.(Lond)* 29 Suppl 1, S36, 2005.
77. Mattson, M.P. Dietary factors, hormesis and health. *Ageing Res. Rev.* 7, 43–48, 2007.
78. Lamming, D.W., Wood, J.G., and Sinclair, D.A. Small molecules that regulate lifespan: Evidence for xenohormesis. *Mol. Microbiol.* 53, 1003, 2004.
79. Hipkiss, A.R. Dietary restriction, glycolysis, hormesis and ageing. *Biogerontology.* 8, 221, 2007.
80. Leakey, J.E., Cunny, H.C., Bazare, J., Jr. et al. Effects of aging and caloric restriction on hepatic drug metabolizing enzymes in the Fischer 344 rat. I: The cytochrome P-450 dependent monooxygenase system. *Mech. Ageing Dev.* 48, 145, 1989.
81. Howitz, K.T., and Sinclair, D.A. Xenohormesis: Sensing the chemical cues of other species. *Cell* 133, 387, 2008.